

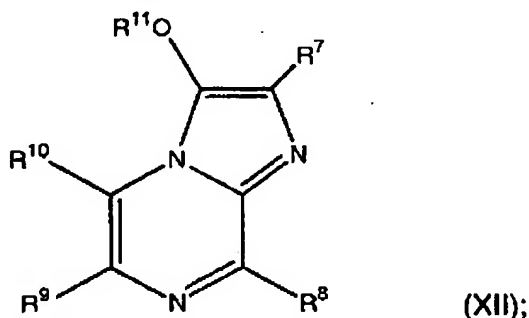
Amendment and Request Continued Examination
Dated October 3, 2005

Appl. No. 10/053,482

Amendments to the Claims

Please amend the claims as follows (the changes in these claims are shown with ~~striketrough~~ for deleted text and underlines for added text). A complete listing of the claims is listed below with proper claim identifiers. This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Currently amended) A compound of formula (XII)



wherein R^7 is H, alkyl, heteroalkyl, aryl, or $-\text{CH}_2-\text{C}_6\text{H}_4\text{OR}^{14}$;

R^8 is H, alkyl, heteroalkyl, or aryl;

R^9 is H, alkyl, heteroalkyl, aryl, or $-\text{C}_6\text{H}_4\text{OR}^{15}$;

R^{10} is -H, $-\text{CH}_3$, or $-\text{CH}(\text{CH}_3)_2$; and

R^{11} , R^{14} , and R^{15} are each independently a protecting group that is removable by an enzyme ~~enzyme-removable groups~~;

with the proviso that R^{11} , R^{14} , and R^{15} are not all acetyl groups.

2. (Original) The compound of claim 1, wherein

R^7 is $-\text{CH}_2-\text{C}_6\text{H}_5$, naphthyl, $-\text{CH}_2-\text{C}_6\text{H}_4\text{OH}$, $-\text{CH}_2-\text{C}_6\text{H}_4\text{F}$, or $-\text{CH}_2-\text{C}_6\text{H}_4\text{OR}^{14}$;

R^8 is $-\text{CH}_2\text{C}_6\text{H}_5$, $-\text{CH}_2\text{C}_6\text{H}_{11}$, $-\text{CH}_2\text{C}_5\text{H}_9$, or $-(\text{CH}_2)_3\text{NHC}(=\text{NH})\text{NH}_2$; and

R^9 is phenyl, indolyl, $-\text{C}_6\text{H}_4\text{OH}$, $-\text{C}_6\text{H}_4\text{NH}_2$, $-\text{C}_6\text{H}_4\text{F}$, or $-\text{C}_6\text{H}_4\text{OR}^{15}$.

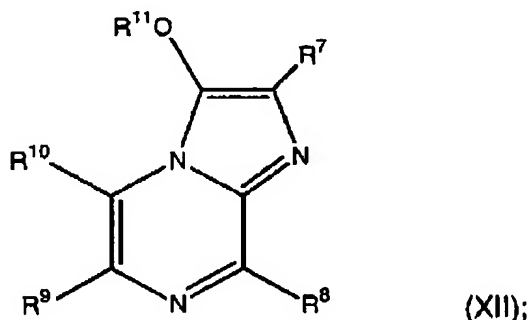
3. (Previously presented) The compound of claim 1, wherein $-\text{OR}^{11}$, $-\text{OR}^{14}$, and $-\text{OR}^{15}$ are each independently esters.

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4. (Original) The compound of claim 1, wherein
 R^{11} is acetyl; and
 R^{14} and R^{15} are independently butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.
5. (Original) The compound of claim 1, wherein
 R^{11} is butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl; and
 R^{14} and R^{15} are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.

6. (Currently amended) A compound of formula (XII)



wherein R^7 is H, alkyl, heteroalkyl, aryl, or $-\text{CH}_2-\text{C}_6\text{H}_4\text{OR}^{14}$;
 R^8 is H, alkyl, heteroalkyl, or aryl;
 R^9 is H, alkyl, heteroalkyl, aryl, or $-\text{C}_6\text{H}_4\text{OR}^{15}$;
 R^{10} is -H, $-\text{CH}_3$, or $-\text{CH}(\text{CH}_3)_2$; and
 R^{11} , R^{14} , and R^{15} are each independently a protecting group that is removable by an enzyme ~~enzyme-removable groups~~; and
 wherein the concentration of the compound in a mixture comprising F12 medium and 10% fetal bovine serum at 22°C is reduced by less than 50% after 45 minutes.

7. (Original) The compound of claim 6, wherein
 R^7 is $-\text{CH}_2-\text{C}_6\text{H}_5$, naphthyl, $-\text{CH}_2-\text{C}_6\text{H}_4\text{OH}$, $-\text{CH}_2-\text{C}_6\text{H}_4\text{F}$, or $-\text{CH}_2-\text{C}_6\text{H}_4\text{OR}^{14}$;
 R^8 is $-\text{CH}_2\text{C}_6\text{H}_5$, $-\text{CH}_2\text{C}_6\text{H}_{11}$, $-\text{CH}_2\text{C}_5\text{H}_9$, or $-(\text{CH}_2)_3\text{NHC}(=\text{NH})\text{NH}_2$; and

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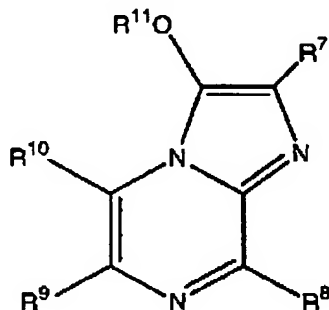
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R^9 is phenyl, indolyl, $-C_6H_4OH$, $-C_6H_4NH_2$, $-C_6H_4F$, or $-C_6H_4OR^{15}$.

8. (Currently amended) The compound of claim 6, wherein R^{11} , R^{14} , and R^{15} $-OR^{11}$, $-OR^{14}$, and $-OR^{15}$ are each independently esters.

9. (Original) The compound of claim 6, wherein R^{11} , R^{14} , and R^{15} are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.

10. (Currently amended) A compound of formula (XII)



(XII);

wherein R^7 is H, alkyl, heteroalkyl, aryl, or $-CH_2-C_6H_4OR^{14}$;

R^8 is H, alkyl, heteroalkyl, or aryl;

R^9 is H, alkyl, heteroalkyl, aryl, or $-C_6H_4OR^{15}$;

R^{10} is $-H$, $-CH_3$, or $-CH(CH_3)_2$; and

R^{11} , R^{14} , and R^{15} are each independently a protecting group that is removable by an enzyme ~~enzyme-removable groups~~; and

wherein the removal of at least one protecting group that is removable by an enzyme ~~enzyme-removable group~~ provides a parent compound; and

wherein the time necessary for the concentration of the compound in a mixture comprising F12 medium and 10% fetal bovine serum at 22°C to be reduced by 50% is greater than the time necessary for the concentration of the parent compound in a mixture comprising F12 medium and 10% fetal bovine serum at 22°C to be reduced by 50%.

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11. (Currently amended) The compound of claim 10, wherein the removal of at least two protecting groups that are removable by an enzyme ~~enzyme-removable groups~~ provides the parent compound.

12. (Currently amended) The compound of claim 10, wherein the removal of all protecting groups that are removable by an enzyme ~~enzyme-removable groups~~ provides the parent compound.

13. (Original) The compound of claim 10, wherein

R^7 is $-\text{CH}_2-\text{C}_6\text{H}_5$, naphthyl, $-\text{CH}_2-\text{C}_6\text{H}_4\text{OH}$, $-\text{CH}_2-\text{C}_6\text{H}_4\text{F}$, or $-\text{CH}_2-\text{C}_6\text{H}_4\text{OR}^{14}$;

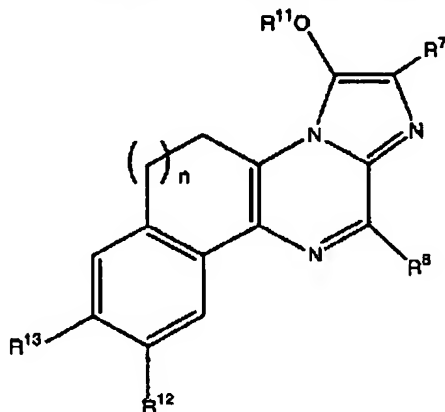
R^8 is $-\text{CH}_2\text{C}_6\text{H}_5$, $-\text{CH}_2\text{C}_6\text{H}_{11}$, $-\text{CH}_2\text{C}_5\text{H}_9$, or $-(\text{CH}_2)_3\text{NHC}(=\text{NH})\text{NH}_2$; and

R^9 is phenyl, indolyl, $-\text{C}_6\text{H}_4\text{OH}$, $-\text{C}_6\text{H}_4\text{NH}_2$, $-\text{C}_6\text{H}_4\text{F}$, or $-\text{C}_6\text{H}_4\text{OR}^{15}$.

14. (Previously presented) The compound of claim 10, wherein $-\text{OR}^{11}$, $-\text{OR}^{14}$, and $-\text{OR}^{15}$ are each independently esters.

15. (Original) The compound of claim 10, wherein R^{11} , R^{14} , and R^{15} are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.

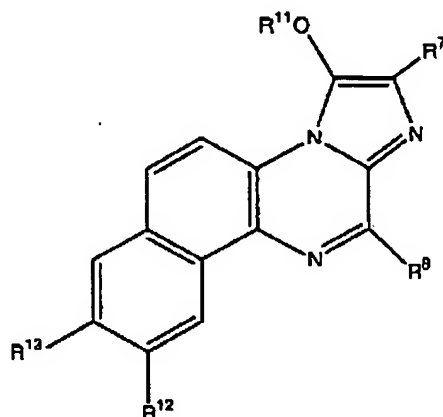
16. (Currently amended) A compound of formula (XIII) or (XIV)



(XIII);

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(XIV);

wherein R^7 is H, alkyl, heteroalkyl, aryl, or $-\text{CH}_2-\text{C}_6\text{H}_4\text{OR}^{14}$;

R^8 is H, alkyl, heteroalkyl, or aryl;

R^{12} and R^{13} are independently -H, -OH, alkyl, heteroalkyl, aryl, or $-\text{OR}^{16}$;

n is 0, 1, or 2; and

R^{11} , R^{14} , and R^{16} are each independently a protecting group that is removable by an enzyme enzyme-removable groups.

17. (Original) The compound of claim 16, wherein R^7 is $-\text{CH}_2-\text{C}_6\text{H}_5$, naphthyl, $-\text{CH}_2-\text{C}_6\text{H}_4\text{OH}$, $-\text{CH}_2-\text{C}_6\text{H}_4\text{F}$, or $-\text{CH}_2-\text{C}_6\text{H}_4\text{OR}^{14}$; and R^8 is $-\text{CH}_2\text{C}_6\text{H}_5$, $-\text{CH}_2\text{C}_6\text{H}_{11}$, $-\text{CH}_2\text{C}_5\text{H}_9$, or $-(\text{CH}_2)_3\text{NHC}(=\text{NH})\text{NH}_2$.
18. (Currently Amended) The compound of claim 16, wherein $-\text{OR}^{11}$, $-\text{OR}^{14}$, and $-\text{OR}^{15}$ are each independently esters.
19. (Original) The compound of claim 16, wherein R^{11} , R^{14} , and R^{16} are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.
20. (Original) The compound of claim 16, wherein n is 1.
21. (Original) A composition, comprising:
the compound of claim 1 in solution.

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22. (Original) The composition of claim 21, wherein the solution is an aqueous solution.
23. (Original) The composition of claim 21, wherein the solution comprises DMSO or alcohol.
24. (Original) A composition, comprising:
the compound of claim 6, in solution.
25. (Original) The composition of claim 24, wherein the solution is an aqueous solution.
26. (Original) The composition of claim 24, wherein the solution comprises DMSO or alcohol.
27. (Original) A composition, comprising:
the compound of claim 10, in solution.
28. (Original) The composition of claim 27, wherein the solution is an aqueous solution.
29. (Original) The composition of claim 27, wherein the solution comprises DMSO or alcohol.
30. (Original) A composition, comprising:
the compound of claim 16, in solution.
31. (Original) The composition of claim 30, wherein the solution is an aqueous solution.
32. (Original) The composition of claim 30, wherein the solution comprises DMSO or alcohol.
33. (Currently amended) A protected luminophore, which is
a ~~modified~~ protected coelenterazine that includes an imidazolone
oxygen protected with a protecting group that is removable by an enzyme;

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wherein the protecting group together with the imidazolone oxygen to which it is attached, form ~~is included in an ester or an ether comprising an enzyme-removable group;~~

wherein subsequent removal of said protecting group ~~enzyme-removable group~~ provides a parent coelenterazine; and

wherein the time necessary for the concentration of the protected modified coelenterazine in a mixture comprising F12 medium and 10% fetal bovine serum at 22°C to be reduced by 50% is greater than the time necessary for the concentration of the parent coelenterazine in a mixture comprising F12 medium and 10% fetal bovine serum at 22°C to be reduced by 50%.

34. (Cancelled) A kit, comprising:
a protected luminophore; and
a luminogenic protein.

35. (Cancelled) The kit of claim 34, further comprising a deprotecting enzyme separate from the luminophore.

36. (Cancelled) The kit of claim 34, wherein the protected luminophore and the luminogenic protein are in separate containers.

37. (Cancelled) The kit of claim 34, wherein the protected luminophore and the luminogenic protein are in the same container.

38. (Withdrawn) A kit, comprising:
a protected luminophore; and
a deprotecting enzyme;
wherein the luminophore and the deprotecting enzyme are in separate containers.

39. (Withdrawn) A method of measuring the enzymatic activity of a luminogenic protein comprising:
contacting a luminogenic protein, a deprotecting enzyme, and a protected luminophore in solution to form a composition; and

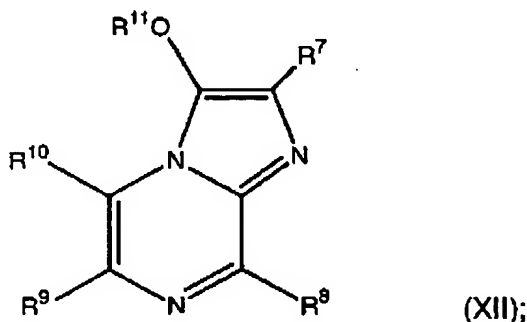
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detecting light produced from the composition.

40. (Withdrawn) The method of claim 39, wherein the luminogenic protein is *Renilla* luciferase.

41. (Withdrawn-Currently amended) The method of claim 39, wherein the protected luminophore is a compound of formula (XII)



wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴;

R⁸ is H, alkyl, heteroalkyl, or aryl;

R⁹ is H, alkyl, heteroalkyl, aryl, or -C₆H₄OR¹⁵;

R¹⁰ is -H, -CH₃, or -CH(CH₃)₂; and

R¹¹, R¹⁴, and R¹⁵ are each independently a protecting group that is removable by an enzyme ~~enzyme-removable groups~~.

42. (Withdrawn) The method of claim 41, wherein
R⁷ is -CH₂-C₆H₅, naphthyl, -CH₂-C₆H₄OH, -CH₂-C₆H₄F, or -CH₂-C₆H₄OR¹⁴;

R⁸ is -CH₂C₆H₅, -CH₂C₆H₁₁, -CH₂C₅H₉, or -(CH₂)₃NHC(=NH)NH₂; and

R⁹ is phenyl, indolyl, -C₆H₄OH, -C₆H₄NH₂, -C₆H₄F, or -C₆H₄OR¹⁵.

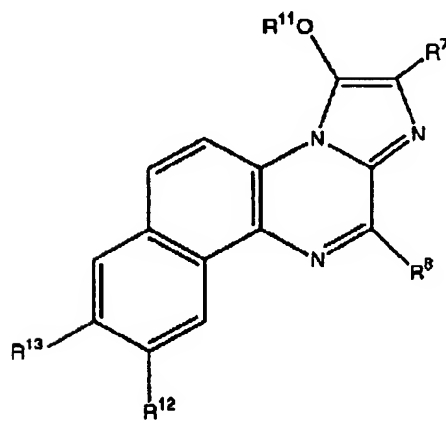
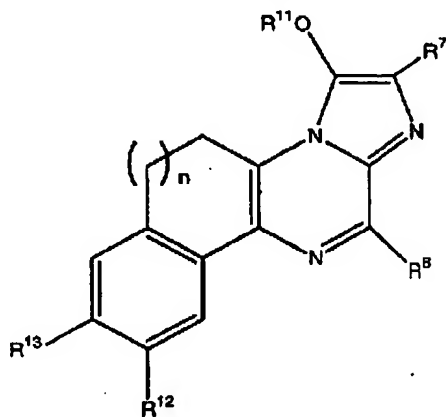
43. (Withdrawn-Currently amended) The method of claim 41, wherein ~~R¹¹, R¹⁴, and R¹⁵~~ -OR¹¹, -OR¹⁴, and -OR¹⁵ are each independently esters.

44. (Withdrawn) The method of claim 41, wherein R¹¹, R¹⁴, and R¹⁵ are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.

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45. (Withdrawn – Currently amended) The method of claim 39, wherein the protected luminophore is a compound of formula (XIII) or (XIV)



wherein R^7 is H, alkyl, heteroalkyl, aryl, or $-\text{CH}_2-\text{C}_6\text{H}_4\text{OR}^{14}$;

R^8 is H, alkyl, heteroalkyl, or aryl;

R^{12} and R^{13} are independently -H, -OH, alkyl, heteroalkyl, aryl, or $-\text{OR}^{16}$;

n is 0, 1, or 2; and

R^{11} , R^{14} , and R^{16} are each independently a protecting group that is removable by an enzyme ~~enzyme-removable groups~~.

46. (Withdrawn) The method of claim 45, wherein

R^7 is $-\text{CH}_2-\text{C}_6\text{H}_5$, naphthyl, $-\text{CH}_2-\text{C}_6\text{H}_4\text{OH}$, $-\text{CH}_2-\text{C}_6\text{H}_4\text{F}$, or $-\text{CH}_2-\text{C}_6\text{H}_4\text{OR}^{14}$; and

R^8 is $-\text{CH}_2\text{C}_6\text{H}_5$, $-\text{CH}_2\text{C}_6\text{H}_{11}$, $-\text{CH}_2\text{C}_5\text{H}_9$, or $-(\text{CH}_2)_3\text{NHC}(=\text{NH})\text{NH}_2$.

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47. (Withdrawn-Currently amended) The method of claim 45, wherein R^{11} , R^{14} , and R^{16} -OR¹¹, -OR¹⁴, and -OR¹⁵ are each independently esters.

48. (Withdrawn) The method of claim 45, wherein R^{11} , R^{14} , and R^{16} are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.

49. (Withdrawn) The method of claim 45, wherein n is 1.

50. (Withdrawn) The method of claim 39, wherein the composition comprises a cell.

51. (Withdrawn) The method of claim 39, wherein the composition comprises a cell which contains the deprotecting enzyme.

52. (Withdrawn) The method of claim 51, wherein detecting light produced from the composition indicates the location of the deprotecting enzyme in a cell.

53. (Withdrawn) The method of claim 39, wherein the composition comprises a cell lysate.

54. (Withdrawn) The method of claim 39, wherein the deprotecting enzyme is an esterase.

55. (Withdrawn) The method of claim 39, wherein the solution is an aqueous solution.

56. (Withdrawn) The method of claim 39, wherein the solution comprises DMSO.

57. (Withdrawn-Currently amended) The method of claim 39, wherein the protected luminophore is a protected ~~modified~~ coelenterazine;

wherein the enol group has been converted to an ester or an ether comprising ~~as a~~ protecting group that is removable by an enzyme ~~enzyme-removable group~~.

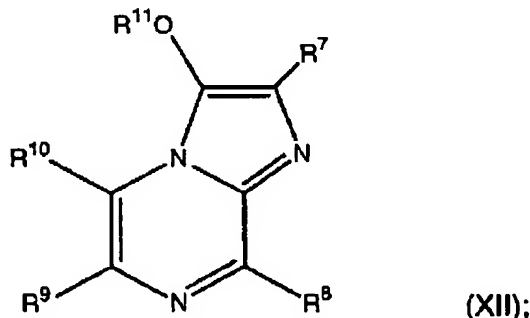
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58. (Withdrawn) A method of generating luminescence in a living cell comprising a luciferase, the method comprising:
contacting the cell in solution with a protected luminophore.

59. (Withdrawn-Currently amended) The method of claim 58, wherein the protected luminophore is a protected modified coelenterazine;
wherein the enol group has been converted to an ester or an ether
comprising an protecting group that is removable by an enzyme ~~enzyme-removable group~~.

60. (Withdrawn-Currently amended) The method of claim 58, wherein the protected luminophore is a compound of formula (XII)



wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴;

R⁸ is H, alkyl, heteroalkyl, or aryl;

R⁹ is H, alkyl, heteroalkyl, aryl, or -C₆H₄OR¹⁵;

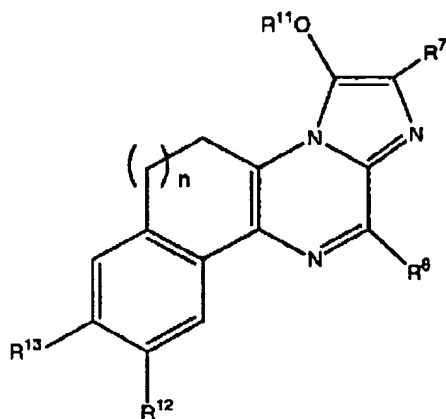
R¹⁰ is -H, -CH₃, or -CH(CH₃)₂; and

R¹¹, R¹⁴, and R¹⁵ are each independently a protecting group that is removable by an enzyme ~~enzyme-removable groups~~.

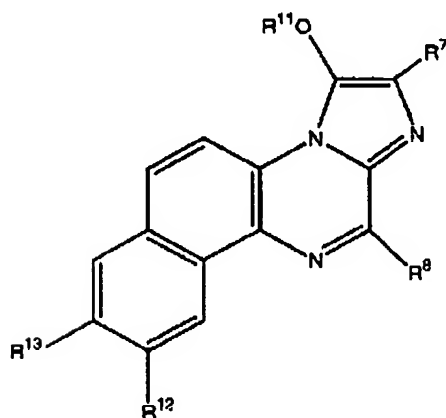
61. (Withdrawn-Currently amended) The method of claim 58, wherein the protected luminophore is a compound of formula (XIII) or (XIV)

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(XIII);



(XIV);

wherein R^7 is H, alkyl, heteroalkyl, aryl, or $-\text{CH}_2-\text{C}_6\text{H}_4\text{OR}^{14}$;

R^8 is H, alkyl, heteroalkyl, or aryl;

R^{12} and R^{13} are independently -H, -OH, alkyl, heteroalkyl, aryl, or $-\text{OR}^{16}$;

n is 0, 1, or 2; and

R^{11} , R^{14} , and R^{16} are each independently a protecting group that is removable by an enzyme ~~enzyme-removable groups~~.

62. (Withdrawn) A method of measuring the enzymatic activity of a non-luminogenic enzyme, comprising:

contacting a non-luminogenic enzyme with a liquid mixture comprising a luminogenic protein and a protected luminophore to form a composition; and
detecting light produced from the composition.

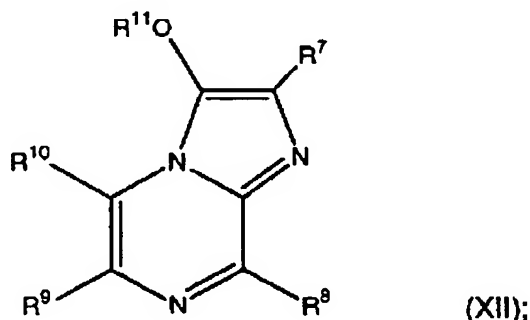
63. (Withdrawn) The method of claim 62, wherein the protected luminophore is a protected ~~modified~~ coelenterazine;

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wherein the enol group has been converted to an ester or an ether comprising an group that is removable by the non-luminogenic enzyme.

64. (Withdrawn-Currently amended) The method of claim 62, wherein the protected luminophore is a compound of formula (XII).



wherein R^7 is H, alkyl, heteroalkyl, aryl, or $-\text{CH}_2-\text{C}_6\text{H}_4\text{OR}^{14}$;

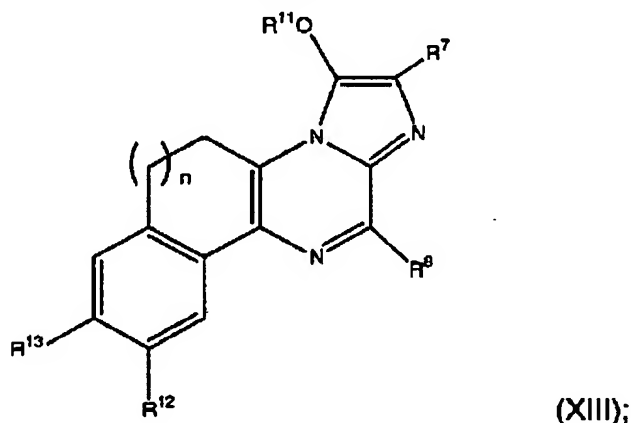
R^8 is H, alkyl, heteroalkyl, or aryl;

R^9 is H, alkyl, heteroalkyl, aryl, or $-\text{C}_6\text{H}_4\text{OR}^{15}$;

R^{10} is -H, $-\text{CH}_3$, or $-\text{CH}(\text{CH}_3)_2$; and

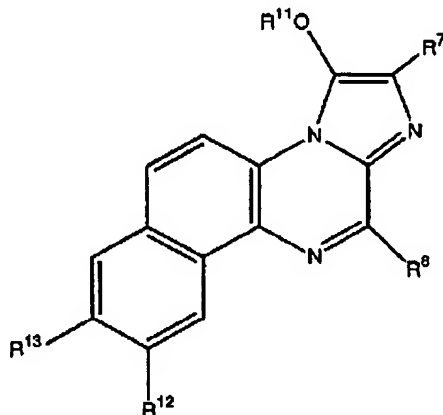
R^{11} , R^{14} , and R^{15} are each independently a protecting group that is removable by an enzyme ~~enzyme-removable groups~~ that are removable by the non-luminogenic enzyme.

65. (Withdrawn-currently amended) The method of claim 62, wherein the protected luminophore is a compound of formula (XIII) or (XIV)



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(XIV);

wherein R^7 is H, alkyl, heteroalkyl, aryl, or $-\text{CH}_2-\text{C}_6\text{H}_4\text{OR}^{14}$;

R^8 is H, alkyl, heteroalkyl, or aryl;

R^{12} and R^{13} are independently -H, -OH, alkyl, heteroalkyl, aryl, or $-\text{OR}^{16}$;

n is 0, 1, or 2; and

R^{11} , R^{14} , and R^{16} are each independently protecting enzyme-removable groups that are removable by the non-luminogenic enzyme.

66. (Cancelled) The kit of claim 34, further comprising DMSO or alcohol or a mixture thereof.

67. (Withdrawn) The kit of claim 38, further comprising DMSO or alcohol or a mixture thereof in the same container as the protected luminophore.

68. (Previously presented) The compound of claim 1, wherein R^{11} , R^{14} , and R^{15} are independently selected from the group consisting of an alkyl group containing from 1-20 carbon atoms and a heteroalkyl group containing from 1-20 carbon atoms.

69. (Previously presented) The compound of claim 1, wherein R^{11} , R^{14} , and R^{15} are independently selected from the group consisting of an alkyl group containing from 1-15 carbon atoms and a heteroalkyl group containing from 1-15 carbon atoms.

70. (Previously presented) The compound of claim 1, wherein

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R^{11} , R^{14} , and R^{15} are independently a heteroalkyl group containing from 1-20 carbon atoms, and wherein $-OR^{11}$, $-OR^{14}$, and $-OR^{15}$ are each independently an ester group or an ether group.

71. (Previously presented) The compound of claim 10, wherein R^{11} , R^{14} , and R^{15} are independently selected from the group consisting of an alkyl group containing from 1-20 carbon atoms and a heteroalkyl group containing from 1-20 carbon atoms.

72. (Previously presented) The compound of claim 10, wherein R^{11} , R^{14} , and R^{15} are independently a heteroalkyl group containing from 1-20 carbon atoms, and wherein $-OR^{11}$, $-OR^{14}$, and $-OR^{15}$ are each independently an ester group or an ether group.

73. (Previously presented) The compound of claim 16, wherein R^{11} , R^{14} , and R^{16} are independently selected from the group consisting of an alkyl group containing from 1-20 carbon atoms and a heteroalkyl group containing from 1-20 carbon atoms.

74. (Previously presented) The compound of claim 16, wherein R^{11} , R^{14} , and R^{16} are independently a heteroalkyl group containing from 1-20 carbon atoms, and wherein $-OR^{11}$, $-OR^{14}$, and $-OR^{16}$ are each independently an ester group or an ether group.

75. (Withdrawn) The method of claim 41, wherein R^{11} , R^{14} , and R^{15} are independently selected from the group consisting of an alkyl group containing from 1-20 carbon atoms and a heteroalkyl group containing from 1-20 carbon atoms.

76. (Withdrawn) The method of claim 41, wherein R^{11} , R^{14} , and R^{15} are independently a heteroalkyl group containing from 1-20 carbon atoms, and comprising at least one of an ester group and an ether group.

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77. (Withdrawn) The method of claim 45, wherein R^{11} , R^{14} , and R^{16} are independently selected from the group consisting of an alkyl group containing from 1-20 carbon atoms and a heteroalkyl group containing from 1-20 carbon atoms.

78. (Withdrawn) The method of claim 45, wherein R^{11} , R^{14} , and R^{16} are independently a heteroalkyl group containing from 1-20 carbon atoms, and comprising at least one of an ester group and an ether group.

79. (New) The compound of claim 1, wherein the enzyme is selected from the group consisting of hydrolases, esterases, phosphatases, phosphodiesterases, glycosidases and proteases.

80. (New) The compound of claim 1, wherein the protecting group is selected from the group consisting of ester, ether, phosphoryl and glucosyl.

81. (New) The compound of claim 5, wherein R^7 is $-\text{CH}_2-\text{C}_6\text{H}_5$, naphthyl, $-\text{CH}_2-\text{C}_6\text{H}_4\text{OH}$, $-\text{CH}_2-\text{C}_6\text{H}_4\text{F}$, or $-\text{CH}_2-\text{C}_6\text{H}_4\text{OR}^{14}$; R^8 is $-\text{CH}_2\text{C}_6\text{H}_5$, $-\text{CH}_2\text{C}_6\text{H}_{11}$, $-\text{CH}_2\text{C}_5\text{H}_9$, or $-(\text{CH}_2)_3\text{NHC}(=\text{NH})\text{NH}_2$; and R^9 is $-\text{C}_6\text{H}_4\text{OR}^{15}$.

82. (New) The compound of claim 1 of the formula:

